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Mycorrhizal Association of *Glomus clarum* with Watermelon and Soybean[1]

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Abstract : The growth of soybean was enhanced by the inoculation with *Glomus clarum* at the initial inoculum of 50 chlamydospores per plant. The invading hyphae of *G. clarum* produced appresoria on the root surface of watermelon and soybean at 5 and 9 days after inoculation, respectively. Following infection, the fungus colonized in cortex. Arbuscules were formed by repeated dichotomous branches of intracellular hyphae until they ended in a mass of fine hyphal branches occupying the cortical cells. Vesicles, which were usually intercellular, were found in cortical cells of watermelon 6 days after inoculation and reached abundance 2 days later. The external mycelium developed from the infection hyphae and produced spores at 10 and 19 days after inoculation of watermelon and soybean respectively. *Paspalum notatum* was suggested as a suitable host plant to increase inoculum of *G. clarum*, and the inoculum production could be as much as 13 times of the initial amount in 8 weeks after inoculation.

Key words : Watermelon, Soybean, Arbuscules, Vesicles, Endomycorrhiza *Glomus clarum*.

INTRODUCTION

Endomycorrhiza was first documented in the late 18th century[21]. Until the advanced experimental techniques were developed in the middle of 1950's, researchers began to study mycorrhizal fungi, and found the beneficial efficiency of these fungi to crops[2,10,11,12,18,22]. Gerdemann[10] indicated that endomycorrhizae were formed in 97% of infected plants. Mycorrhizal fungi were distrbuted worldwidely from North to South poles[10,17].

In the previous studies[3], we found that arbuscular mycorrhizal fungi were widely distrbuted in the soybean fields in Taichung, Huwei and Pingtung areas. The density of arbuscular mycorrhizal fungi in the fields was 5 to 148 spores per 200 gram of soils. The most common mycorrhizal fungus was *Glomus clarum* Nicol. & Schenck which sporulated abundantly in field in spring and autumn. The second was *Acaulospora laevis* Gerd.

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& Trappe in summer. *Gigaspora margarita* Becker & Hall, *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe and *G. macrocarpum* Tul. & Tul. were also occasionally isolated from soybean fields. The chlamydospores of *G. clarum* were transparent or dark yellow, spherical or ovary, and ranged from 85-250 μm (av. 150 μm) in diameter. The growth and pod production of soybean seedling infected by *G. clarum* increased[4,5]

In this study, we reported the infection process of *G. clarum* in soybean and watermelon, and studied the effective inoculum of this fungus for field applecation. Besides, suitable hosts for mass production of the inoculum of *G. clarum* were also suggested.

MATERIALS AND METHODS

1. Inoculum production of vesicular-arbuscular mycorrhizal fungus (VAMF)

Glomus clarum is the most prevalent VAM fungus distributed in the soybean fields in Taiwan. Chlamydospores of this fungus were collected by the method of wet sieving and decanting[9,13,20]. Soils with roots taken from the rhizospheres of well-growing soybean plants were homogenized in distilled water and followed by passing through 250-μm and 100-μm sieves. Materials retained on 60-μm sieves containing VAMF chlamydospores were resuspened in distilled water and examined under disecting microscope. After surface disinfection with 0.05% sodium hypochloride for 2 min and washed in sterile distilled water twice, chlamydospore suspension (200 spores per pot) was pippeted onto the roots of *Paspalum notatum* Flugge seedling grown in pot containing pasteurized sandy loam soils. Pot soil was autoclaved at 100°C for one hour previously. Controls were inoculated with the chlamydospores-free filtrates of *G. clarum*. Inoculated seedlings were then moved to greenhouse where the temperature was maintained at 24-30°C.

For examining mycorrhizae, roots taken from tested crops were washed and cut into 2 -5 cm long pieces. Root fragments were then heated on hot plate in 10% KOH solution at 95 °C for 3 min. After cooling, root fragments were rinsed with distilled water 3 times, and 10 min later, roots were immersed into 0.05% trypan blue in lactophenol for 2 hours. The destained roots by tap water were then examined by microscope[19]. Infection rates (%) were counted by examining 100 root fragments for each treatment.

2. Determination of the density of effective inoculum of *G. clarum* for host plants

This study was conducted in glasshouse at 24-30°C. Soybean seedlings (Tainon No. 15) used as test plants grew in pots (12 cm in diameter) containing sterile sandy loam soil. The sandy loam soil (pH 8.1) containing 0.64% organic matter, 366 Kg/ha P_2O_5 and 181 Kg /ha K_2O, was collected from an upland crop field at Kueilen, Tainan. The soil was pasteurized at 100°C for one hour before use. Each 5-day-old soybean seedling in one pot was inoculated with 50,100,200,400, 800, or 1600 chlamydospores of *G. clarum* into the rhizosphere soil, respectively. Six pots were used in each treatment. Controls were inoculated with chlamydospore-free filtrate. Seedling growth was measured weekly. After growth for 8 weeks, seedling roots were examined for the mycorrhizal formation.

3. VA mycorrhiza of *Glomus clarum*.

7-day-old seedlings of soybean and watermelon (Kinglan) were inoculated with 100

chlamydospores of *G. clarum* and transferred into a root-zone growth chamber at 28°C. Roots of seedlings were harvested daily and stained with lactophenol trypan blue to examine the infection procedure of VAM fungus.

4. Examination of VA mycorrhiza by scanning electron microscope

Mycorrhizal roots of soybean or watermelon seedling were cut into 5 cm pieces and then fixed, dehydrated, and coated by gold for ultrastructural observation following the procedures as described by Dewes[8]. Busch & Lomb scanning electron microscope was operated in this study.

RESULTS

Two-week-old seedings of *P. notatum* inoculated with *G. clarum* grew faster than controls (Fig. 6-A). Arbuscules and vesicles were formed in the cortex cells of host roots at 10 days after inoculation (Fig. 3 & 4). After inoculation for 8 weeks, chlamydospores of *G. clarum* increased to about 180 spores per 100 g soils as much as 13 times of the initial inoculum.

Results in Table 1 showed that *G. clarum* was effective in promoting plant growth and increasing pod yield of soybean Tainon No. 5 at the initial density of inoculum of 50 to 1600 chlamydospores per seedling. After inoculation for 8 weeks, the average height of soybean plants were 37.9, 39.5, 41.6, 39.2, 40.2, and 39.0 cm for treatments in which 50, 100, 200, 400, 800, and 1600 spores were inoculated respectively. The controls were 34.1 cm high in average. The root growth and biomass of mycorrhizal soybeans were also better and heavier than those of non-mycorrhizal controls. The average pods produced by mycorrhizal soybeans were 8 to 9, while non-mycorrhizal controls produced only 5 pods. Colonization rate for mycorrhizal plants were 38 to 57%.

Table 1. Effect of inoculum density of *Glomus clarum* on the growth of soybean [z]

Inoculum density of *G.clarum* (Spores/pot)	Average growth of soybean				% of root endomycorrhiza	Pods/plants
	Stem height (cm)	Stem weight (g)	Root length (cm)	Root weight (g)		
50	37.8 ab[y]	17.5 a	27.3 a	10.1 a	38	8.3
100	39.5 ab	17.1 a	27.5 a	10.6 a	42	9.6
200	41.6 a	19.0 a	28.5 a	11.1 a	52	9.8
400	39.2 ab	16.3 a	27.8 a	9.6 a	50	8.6
800	40.2 ab	16.9 a	28.6 a	10.5 a	51	8.7
1,600	39.0 ab	16.4 a	30.5 a	11.2 a	57	8.5
CK (0)	34.1 b	12.0 b	23.5 b	8.2 a	0	5.8

[z] Each value is the mean of 6 plants.
[y] Data with the same letter do not differ significantly at 5% level under Duncan's multiplerange analysis.

Some runner hyphae developed from germinating chlamydospores of *G. clarum* formed appressoria on the epidermal cells of watermelon roots at the 5th day of inoculation (Fig. 1-B). Penetration hyphae were observed to penetrate the epidermal cells directly or grow from the intercellular spaces to reach the cortex cells. After enter cortex cells, mycelia of *G. clarum* pentrated to 3 layered cells and moved parallely in cortex cells rapidly. Chains of arbuscules formed from repeated branching of intracellular mycelia were observed in longitudinal sections of roots (Fig. 3). The appressoria then produced infection hyphae penetrating epidermal cells and reached cortical cells. However, some of germinating hyphae directly penetrating epidermal cells without forming appressoria (Fig. 1-C). The intra- or intercullular hyphae of *G. clarum* then penetrated the adjacent cortex cells (Fig. 2-A). The hyphae of *G. clarum* within cortical cells were branched dichotomously and repeatedly, and eventually ended numerous finest hyphae (Fig. 2-B) which were called arbuscules [16] (Fig. 2-C,D). Some finest hyphae might expand into vesicles inside or between the cortical cells. Large amount of arbuscules (Fig. 3) and vesicles (Fig. 4) in the cortex cells were visible mostly at the 6th and 8th day of inoculation separately. Meanwhile, the external hyphae (Fig. 5) and chlamydospores were developed at the 10th day of inoculation (Fig. 5). At 6 days after inoculation, intracellular hyphae began to produce terminal vesicles. At the same time, the external hyphae grew out of the root cells and enter the rhizospheres to form mycelial mats. Chlamydospores were formed by the external hyphae at 10 days after inoculation (Fig. 5).

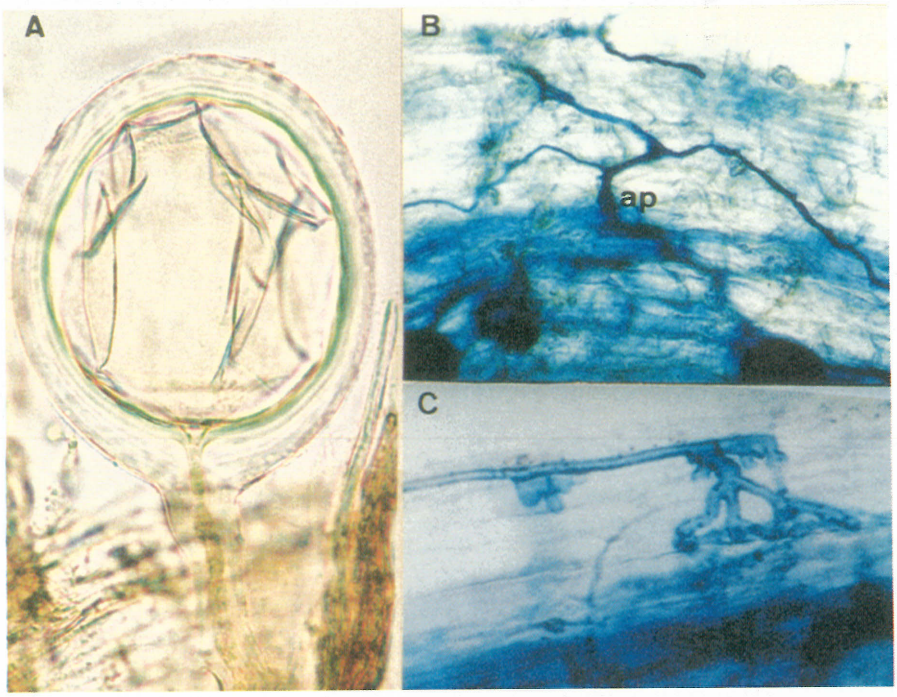

Fig. 1. The germinating chlamydospore penetrated through the cortex cells of watermelon. A. chlamydospore of *Glomus clarum*. B. Formation of appressorium (ap) at the infection site. C. Penetration of hyphae with branching.

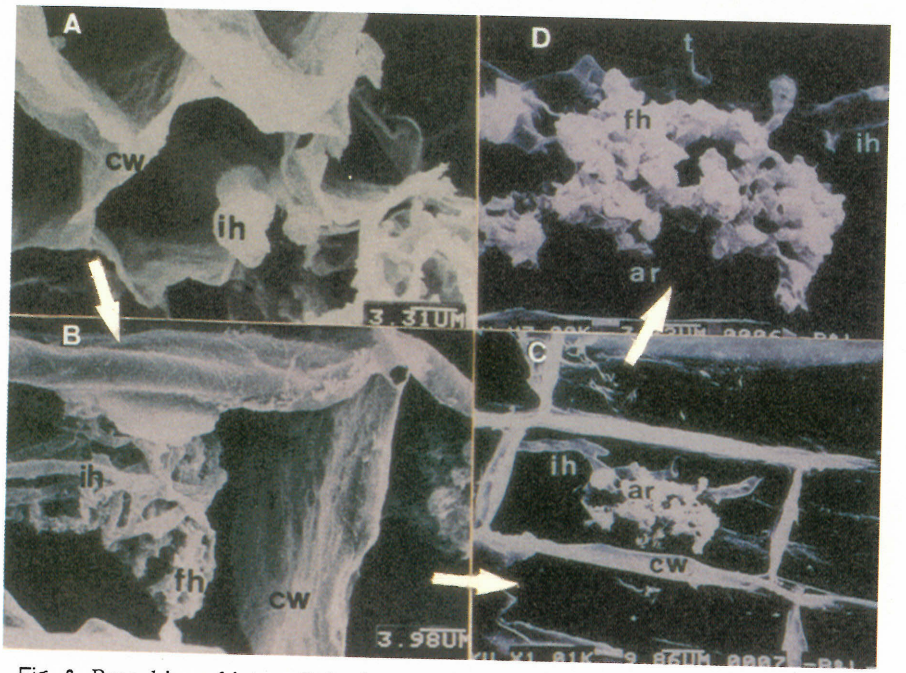

Fig. 2. Branching of intercellular hyphae and development of arbuscules (ar) of *Glomus clarum* in cortex cells of watermelon.

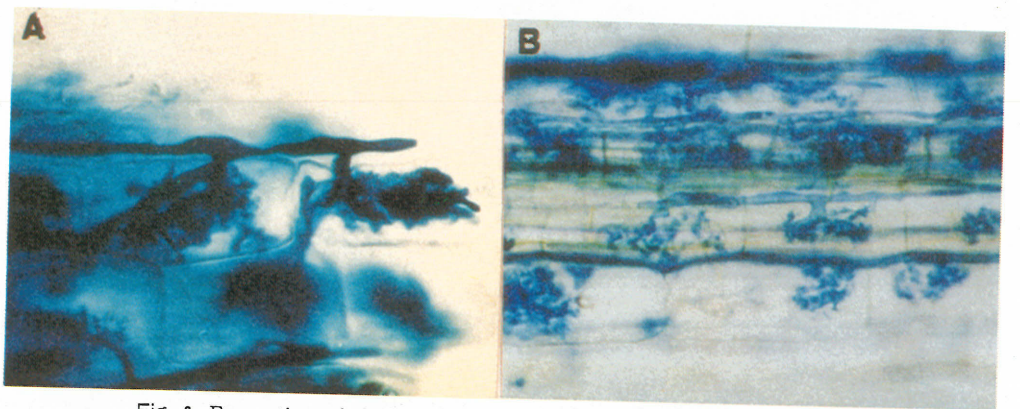

Fig. 3. Formation of chains of arbuscules in cortex cells of watermelon.

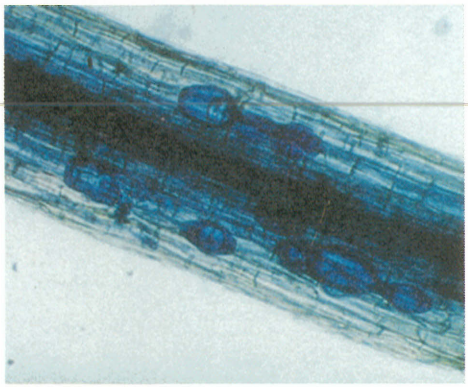

Fig. 4. Formation of vesicles in cortex cells.

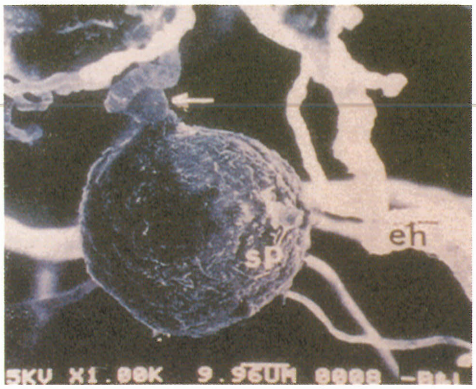

Fig. 5. Development of external hyphae and production of terminal chlamydospore.

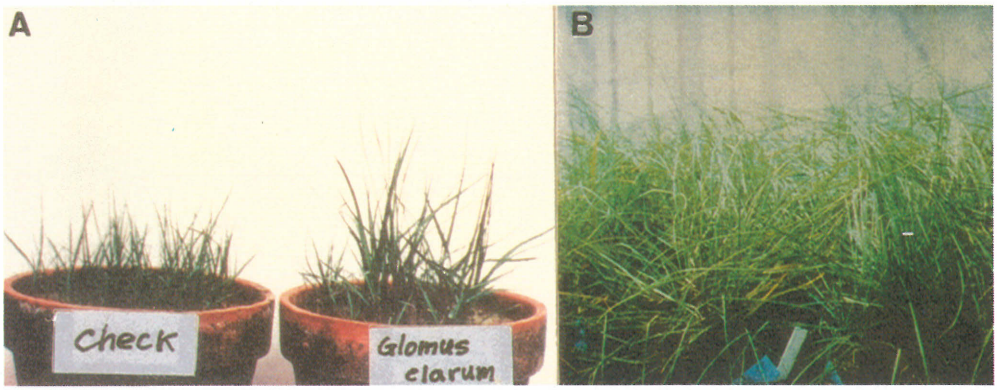

Fig. 6. Cultivation of *Glomus clarum* with bahia grass. A. Endomycorrhizal (Right) and nonmycorrhizal plants (left). B. Mass production of *Glomus clarum* in pot culture.

The infection process and mycorrhizal formation of *G. clarum* in soybean were similar to those in watermelon, but mycorrhiza developed slower in the former. In soybean, infection of *G. clarum* occurred at 9 after inoculation, arbuscules were formed in cortex cells at 11 days and vesicles were formed at 17 days. Fungus produced external hyphae and sporulated at 19 days. This result revealed that the same VAM fungus completed the infection and mycorrhizal formation in different speed when it infected different host plants. Whether it was easier for *G. clarum* to infect fleshy roots of watermelon than woody roots of soybean or some reasons else, it needed more researches to prove.

The mycorrhizal infection of *G. clarum* in soybean was similar to that of watermelon, but the infection process in soybean was longer than that in watermelon. The infection began at the 9th day of inoculation. Arbuscules and vesicles appeared in cortex cells at the 11th and 17th day, respectively, and chlamydospores produced by external hyphae were observed at the 19th day.

DISCUSSION

Vesicles in VA mycorrhizae were generally thought to work in capacity of nutrient storage and form chlamydospores for reproduction[1,7]. In early developing stage, vesicles contained diluted cytoplasm, several oil droplets and glycogen granules[14,15]. Following its development, cytoplasm became condensed and formed many spherical oil drops, which was similar to the contents of *G. clarum* chlamydospores. When vesicles were mature, they pressed against the cortex cells and finally destroyed the epidermal cells to expand out of roots. All of the developing procedures of vesicles were observed in our studies. The developing process of vesicles was thought to have close relations with chlamydospores formation[6].

Before VAM fungi are inoculated to certain crop, researchers would like to know the minial concentration of initial inocula which shows growth promotion effect to the crop. Effective density of inoculum was more important in short-term crops application than long -term crops. In this study, 50 chlamydospores per soybean seedling was enough for growth promotion. When primary inoculum increased up to 200 chlamydospores per plant, the efficacy of *G. clarum* on the seeding growth promotion could be demonstrated more significant. However, the growth promotion was not positively related to the inoculum amount.

Before the artificial media were developed, search for appropriate host plants and culture media for mass production of VAM inoculum are the most important works at this moment. *Paspalum notatum* is considered as one of good hosts for propagation of *G. clarum.* In this study, chlamydospores of *G. clarum* could be increased up to 13 times of the initial inoculum at 8 weeks after inoculation. Since *P. notatum* is a pleiocyclic rhizocarpous grass, it could be maintained in pots for long period.

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內生菌根菌 *Glomus clarum* 在西瓜、大豆之感染過程及菌根之形成[1]

程永雄 杜金池[2]

摘 要

內生菌根菌（*Glomus clarum*）之接種源係採百喜草盆栽培養之土，以篩網傾注法來分離厚膜孢子。內生菌根產生有益效應之最初接種量（initial inoculum density）之決定，是於盆栽大豆進行孢子接種試驗，大豆之最初接種量以每棵50個孢子即能促進植株生長，而每棵接受200個孢子更顯著地的增加地上部及地下部之生長。內生菌根菌 *G. clarum* 之感染過程及菌根形成，分別以大豆及西瓜進行接種試驗，每日採集根部依組織透化法染色根段，以光學顯微鏡及掃描式電子顯微鏡調查眞菌之感染過程。在接種後五天西瓜根部表面上之內生菌根菌之孢子產生發芽管或侵入菌絲末端膨大形成吸盤（appressorium）再產生侵入菌絲，而大豆則在九天後才發生感染（28°C±2°C），侵入菌絲通常貫穿二層細胞，繼之在皮層細胞內發展形成叢枝狀體（arbuscules）。西瓜於接種後 6 天在皮層細胞間出現囊狀體（vesicles），至第 8 天出現多量之囊狀體，此時隨之發展根外之菌絲體，並於接種後第10天在外菌絲末端產生厚膜孢子，而大豆之內生菌根則存接種後第19天才產生厚孢子。

關鍵詞：西瓜、大豆、叢枝狀體、囊狀體、內生菌根菌。

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